PM 1696 - REVISED CLAIMS FOR FOREIGN FILING

1. A recombinant DNA molecule encoding a tobacco protein characterized by the ability to catalyze the transfer of the methyl group of S-adenosylmethionine to the delta amino group of putrescine.

2. A recombinant DNA molecule according to claim 1 encoding a putrescine N-methyltransferase having the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:3.

3. A recombinant DNA molecule according to claim 1 comprising the nucleotide sequence of the cDNA insert of plasmid PMT14-3; or comprising a nycleotide sequence that hybridizes to the said DNA insert or a nucleotide sequence that is degenerate to either of the said nucleotide sequences.

4. A recombinant DNA molecule according to claim 1 comprising the nucleotide sequence or SEQ ID NO:17 or a DNA sequence complementary to such nucleotide sequence.

5. A vector comprising a DNA molecule according to Claim 2, 3 or 4 operably linked to sequences capable of directing the transcription of a mRNA encoded by the isolated DNA molecule.

6. A vector according to claim 5 characterized by a DNA sequence encoding a complementary antisense mRNA linked to sequences capable of directing the transcription of such antisense mRNA.

7. A cultured transgenic tobacco cell stably transformed with a vector according to Claim 5 or 6.

8. A transgenic tobacco plant stably transformed with a vector according to Claim 5 or 6.

9. The putrescine N-methyltransferase encoded by SEQ ID NO:17.

10. The putrescine N-methyltransferase encoded by SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3.

deposited in the American Type Culture Collection, Rockville, Maryland. The culture identified as Escherichia coli, PMT14-3 was deposited on March 11, 1993 and given the ATCC Designation 69253.

Nicotiana tabacum leaves were inoculated with *Agrobacterium tumefaciens* containing the DNA sequence of SEQ ID NO:17, either in the 5' to 3' orientation or the 3' to 5' orientation as follows:

pBI121:

1.2A3-38:

1.2A2-40:

1.2S3-15:

1.2S4-16:

Calluses were grown to plants in a greenhouse and leaf samples were taken. Alkaloids in the leaf samples were extracted from transformed and control leaves, with methanolic potassium hydroxide containing an internal standard. The supernatant was filtered and subjected to gas chromatographic analysis using a nitrogen-phosphorus detector (NPD) to analyze for nicotine and the minor alkaloids, nornicotine, myosmine, anabasine, and anatabine. The following results were obtained:

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Sample	% Nicotine Dry Wt. Basis	% Reduction** in Final Nicotine (Sample Post Top/Control Post Top)	Δ% Nicotine (Post Top minus Pre Top)	% Reduction*** in Δ% Nicotine (Sample Post Top minus Pre Top/Control Post Top minus Pre Top)
pBI121 Controls*			2.544 ± 0.688	
Pre	0.508 ± 0.131			
Post	3.052 ± 0.712			
1.2A3-38				
Pre	0.540	65	0.537	79
Post	1.077			
1.2A2-40				
Pre	0.579	45	1.099	57
Post	1.678			
1.2S3-15				
Pre	0.910	38	0.972	62
Post	1.882			
1.2S4-16				
Pre	0.623	43	1.130	56
Post	1.753			

* N=14

** These values represent the % reduction of final nicotine levels as compared to the controls (dry weight basis).

*** These values represent the % reduction in the change (increase) between Pre- and Post- Topping nicotine levels.

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